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(54) **Novel antibiotic 76-11, process for the production thereof, anticoccidiosis agent and domestic animals growth accelerator comprising the same as an effective ingredient.**

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(73) Proprietor: **KAKEN PHARMACEUTICAL CO., LTD.**
No. 28-8, 2-chome, Honkomagome Bunkyo-Ku
Tokyo 113 (JP)
(73) Proprietor: **RIKAGAKU KENKYUSHO**
2-1 Hirosawa
Wako-shi Saitama-ken (JP)

(72) Inventor: **Isono, Kiyoshi**
5-14-13, Nobidome
Niiza-shi Saitama-ken (JP)
Inventor: **Nakamura, Goto**
1-9-22, Kishi-machi
Urawa-shi Saitama-ken (JP)
Inventor: **Fujita, Shigeo**
4-1-7-305, Sakura-josui
Setagaya-ku Tokyo (JP)

(74) Representative: **Vossius Vossius Tauchner Heunemann Rauh**
Siebertstrasse 4 P.O. Box 86 07 67
D-8000 München 86 (DE)

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Description

This invention relates to an antibiotic, a process for the production thereof, an anticoccidiosis agent and a growth accelerating and feed efficiency increasing agent for domestic animals and fowls comprising the antibiotic as an effective ingredient and more particularly, this invention relates to a novel antibiotic 76—11, a process for the production thereof which comprises culturing an antibiotic 76—11 producing microorganism belonging to the genus *Actinomadura* and isolating the antibiotic; an anticoccidiosis agent comprising the antibiotic as an effective ingredient; a growth accelerating and feed efficiency increasing agent for domestic animals and fowls comprising the antibiotic as an effective ingredient; a method for preventing and treating a coccidiosis; a method for accelerating the growth of domestic animals and increasing feed efficiency; and feed for domestic animals and fowls comprising the antibiotic.

The inventors of the present invention, for the purpose of searching for a new useful antibiotic, isolated a microorganism from soil collected in various places and studied an antibiotic produced by the microorganism. As a result, the inventors found that a new antibiotic 76—11 which has never been known in any published literature, was produced by a microorganism belonging to the genus *Actinomadura* and was accumulated in the cells and culture medium.

Coccidiosis is an infectious disease of domestic fowls caused by *Protozoa* belonging to the genus *Eimeria*, giving fowls scours and poor intake of nutrition and finally causing death.

Oocyst which is the first generation of *Protozoa* is excreted with droppings, forms spores and infects fowls one after another. Typical Protozoa described above include *Eimeria tenella, Eimeria acervulina, Eimeria necatrix, Eimeria brunetti, Eimeria maxima*, and so on. Fowls infected with *Protozoa* lose their commercial value. Therefore, the prevention of coccidiosis is a matter of industrial importance. Accordingly, various kinds of preventive and curative means have heretofore been proposed and widely studied. Proposed agents include arsenic, nitrofurane, or bisphenol compounds, sulfa drug, thiazine, quinoline, pyridine or guanidine derivatives, and so on. However, these agents are not effective enough and, further, some new protozoans having a resistance to these agents appear. Accordingly, a new effective agent was necessary.

Under these conditions, the inventors have conducted a search for a medical agent effective against coccidiosis of fowls and found that the antibiotic 76—11 is extremely effective.

It has widely been the practice to add some antibiotics to feed in order to accelerate the growth and increase egg-laying of domestic animals and fowls. However, due to the administration to animals of antibiotics common to man and animals, resistant strains appear which it is feared may have harmful effects on humans. Further, it is feared that humans might take in the antibiotic administered and accumulated in the animal body when eating the meat or the products from the animal concerned.

The inventors studied growth accelerating agents having none of these disadvantages and found that the administration of the antibiotic 76—11 to domestic animals or fowls accelerates the formation of propionic acid in digestive organs and inhibits the increase in viscosity of rumen liquid. On the other hand, it is known that propionic acid is superior to acetic or butyric acids in the coefficient of energy utilization of volatile fatty acid in a living animal body. From the facts described above, the inventors demonstrated that the antibiotic 76—11 would be an excellent agent being capable of accelerating the growth of domestic animals and fowls and increasing feed efficiency thereof.

It is an object of this invention to provide a novel microorganism which is capable of producing the antibiotic 76—11.

Another object of this invention is to provide the new antibiotic 76—11.

Another object of this invention is to provide a method for producing the antibiotic 76—11.

A further object of this invention is to provide an effective agent against coccidiosis of domestic fowls.

Another object of this invention is to provide an excellent agent capable of accelerating the growth of domestic animals and fowls and increasing feed efficiency thereof.

A still further object of this invention is to provide a method for preventing and treating coccidiosis of domestic fowls.

Another object of this invention is to provide a method for accelerating the growth of domestic animals and fowls and increasing feed efficiency thereof.

Yet another object of this invention is to provide a feed for fowls that is effective in preventing and treating coccidiosis.

A final object of this invention is to provide a feed for domestic animals and fowls, capable of accelerating their growth and increasing feed efficiency.

These objects of this invention can be achieved by the new antibiotic 76—11 which is produced by culturing a microorganism capable of producing the antibiotic 76—11, such as the genus *Actinomadura* 76—11 (hereinafter referred to as "Sp. 76—11"), and isolating the antibiotic 76—11 from the culture liquid.

Figure 1 shows an ultraviolet absorption spectrum of the antibiotic 76—11 (free acid) of the invention;

Figures 2 and 3 show infrared absorption spectra of the antibiotic 76—11 in the form of Na salt and free acid (in KBr plate), respectively.

The new antibiotic 76—11, the novel microorganism capable of producing it and process for the production thereof will now be described in detail.

The microorganisms used in the process of this invention belong to the genus *Actinomadura* and are

capable of producing the antibiotic 76—11. One xample of the microorganisms is Sp. 76—11 which belongs to the genus *Actinomadura* and has the microbiol gical pr perties described below. Not only natural and artifical mutants f Sp. 76—11 but also all the species belonging to the genus *Actinomadura* and capabl of producing the antibiotic 76—11 may be used in this invention. The Sp. 76—11 which is one f the subj ct matters of the invention was deposited in accordance with Rul 28 EPC and the Budapest Treaty with the Fermentation Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan, International Depositary Authority (hereinafter referred to as "FERM" under the accession number FERM BP-83).

The properties of Sp. 76—11 on various culture media were observed 20 to 30 days after inoculation. Color tones described in parentheses are based on the Description Color Name Dictionary.

(I) Morphology

Sp. 76—11 grows on oatmeal agar and malto extract-yeast extract agar culture media but does not or hardly grows on other ones and, therefore, the morphology on oatmeal agar, malto extract-yeast extract agar, and 3% oatmeal liquid culture media was observed. The results are as follows

(i) Substrate mycelia

Substrate mycelia stretch and ramify on both the agar and the liquid culture media. After a long period of culturing, substrate mycelia divide to form elliptical spores the sizes of which are 0.8—1.2×1.5—1.7 μ. Aerial mycelia are not formed on various culture media but, when cultured on oatmeal agar culture medium at 33°C for more than twenty days, white, thin aerial mycelia are sometimes formed, which are bent and string-like or fasciculate, have a few branches and form no spores.

(ii) Aerial mycelia

Not formed on various culture media but sometimes formed on oatmeal agar culture medium when cultured for more than 30 days. The aerial mycelia formed are irregularly bent. No spores are observed under an electron microscope.

(II) Cell composition

Sp. 76—11 was cultured on the culture medium comprising 1% glucose, 1% yeast extract and 0.1% oatmeal at 33°C for 7 days with shaking. Cells were collected and washed to give a sample for analysis of cell composition. Diamino pimelic acid and sugar composition were analyzed. Meso type of diamino pimelic acid, galactose and madurose were detected.

(III) Growth on various culture media

(i) Sucrose nitrate agar culture (Czapeck's agar culture):
Growth: Very poor. Slight growth observed after 30 days of culturing. The colony is transparent. The surface is light brown (2 ea) and the back is light ivory (2 ca).
Aerial mycelia: Not formed.
Soluble pigment: Not formed.

(ii) Glucose asparagine agar culture:
No growth observed.

(iii) Glycerin asparagine agar culture:
No growth observed.

(iv) Inorganic salt starch agar culture:
Growth: Very poor. Slight growth observed after 30 days. The surface is light yellow brown (3 gc) and the back is yellow brown (3 ie).
Aerial mycelia: Not formed.
Soluble pigment: Not formed.

(v) Tyrosine agar culture:
Growth: Very poor. The surface is light yellow (3 ca) and the back is light ivory (2 ca).
Aerial mycelia: Not formed.
Soluble pigment: Not formed.

(vi) Nutrient agar culture:
Growth: Very poor. After 30 days, both the surface and the back are light ivory (2 ca).
Aerial mycelia: Not formed.
Soluble pigment: Not formed.

(vii) Yeast extract-malto-extract agar culture (ISP No. 2):
Growth: Good. The surface resembles a furrowed, hard coating, and both th surface and the back are light brown (2 ne), sometimes bluish (10 ie).
Aerial mycelia: Slightly white aerial mycelia are formed at times after a long period of culturing.
Soluble pigment: Not formed.

(viii) Oatmeal agar culture (ISP No. 3):
Growth: Good. The surface is smooth, coating-like and blue-indigo (10 ie) and the back is white, finally blue-indigo (10 ne).
Aerial mycelia: White aerial mycelia are formed at times after 30 days.
S luble pigment: N t formed.

(ix) Peptone yeast-extract iron agar culture (ISP. No. 6):
No growth is observed.

(x) Skim milk (37°C)
Growth: Slow, Coagulated and peptonized.

(xi) Glucose peptone gelatin culture (20°C):
Growth: Very slow. Liquidization is observed.

(IV) Physiological properties

(i) Optimum temperature for growth:
27 to 37°C, most preferably 33 to 37°C.

(ii) Liquidization of gelatin: yes

(iii) Hydrolysis of starch: no

(iv) Skim milk:
Coagulated and slightly peptonized.

(v) Formation of melanin-like pigment: no

(vi) Resistance to acid: Acid resistant

(V) Utilization of various carbon sources:
Sp. 76—11 was cultured on Pridham and Gottlieb agar culture (ISP No. 9) (produced by Difco Co.) containing various sugars but no growth was observed and therefore the culture medium described above, to which 0.1% yeast extract was added, was used instead., The results obtained are as follows:

L—Arabinose	+++
D—Xylose	+++
D—Glucose	+++
D—Fructose	+++
Sucrose	+++
L—Inositol	++
L—Rhamnose	+++
Raffinose	++
Mannitol	++++
Control	±

Note: ++++: Very good growth
+++: Good growth
++: Growth
±: Control

Characteristics of Sp. 76—11
In brief, Sp. 76—11 may be characterized by:

(i) Morph logy:

Sp. 76—11 do s not generally form aerial mycelia, but sometimes forms them after a l ng peri d of culturing. Substrat mycelia are bent, and in the latter peri d of culturing divide to f rm elliptical spor s. The mycelia are gram-positive and acid resistant.

(ii) Growth on various media:

Sp. 76—11 does not or hardly grows on any various agar culture media except oatmeal agar and yeast extract-malto extract agar culture media.

The strain grows best on oatmeal agar culture medium and forms blue-indigo, water insoluble pigment in the cell after 3 to 4 weeks of culturing.

(iii) Physiological properties:

The strain grows well at 33 to 37°C, liquidizes gelatine slightly, coagulates and peptonizes skim milk, and forms no melanin pigment.

(iv) Utilization of sugar:

The strain utilizes well any of L-arabinose, D-xylose, D-glucose, D-fructose, sucrose, L-inositol, L-rhamnose, raffinose and mannitol on Pridham and Gottlieb agar culture medium to which 0.1% yeast extract was added.

As described above, Sp. 76—11 seems to be a strain belonging to the genus *Actinomadura* of *Actinomycetes* from the morphology of the spores and mycelia, growth on various media and cell-wall composition. However, comparing the characteristics of Sp. 76—11 with those of strains belonging to the genus *Actinomadura*, described in Nonomura, H. & Y. Ohara: Distribution of actinomycetes in soil, XI, Some new species of the genus Actinomadura, Lechevalier et al., J. Ferment. Technol. 49: 904—912, 1971; Preobrazhenskaya, T. P.; M. A. Sveshnikova & L. P. Terekhova: Key for identification of the species of the Genus Actinomadura. The Biology of the Actinomycetes & Related Organisms 12:30—38, 1977, no strains which produce blue-indigo pigment in cell on oatmeal agar culture medium as Sp. 76—11 does, are described therein.

Accordingly, it was concluded that Sp. 76—11 is a new strain belonging to the genus *Actinomadura*.

In producing the antibiotic 76—11, an antibiotic 76—11-producing microorganism may be cultured according to a conventional method used in the production of antibiotics. The culturing mode is not particularly critical and either liquid culturing or solid culturing may be adopted. In order to perform culturing on an industrial scale and economically, it is advisable to adopt a method in which a culture medium is inoculated with a spore suspension or culture medium of an antibiotic 76—11-producing microorganism and the culturing is carried out with aeration and agitation.

The nutritive source that is used in the present invention is not particularly critical, but any of the nutritive sources customarily used for culturing microorganisms may be used. There may be used starch, dextrin, glycerin, glucose, sucrose, galactose, inositol, mannitol, etc. as carbon sources and oatmeal, yeast, peptone, soybean powder, meat extract, rice bran, wheat bran, urea, corn steep liquor, ammonium salts, nitrates and the other organic or inorganic nitrogen compounds as nitrogen sources respectively. If desired, other inorganic salts, such as sodium chloride, phosphates, metal salts of potassium, calcium zinc, manganese and iron may be added. Animal, vegetable or mineral oils may also be added to the medium, if needed. The culture conditions such as temperature and time may be suitably selected for maximum production of the antibiotic 76—11. The culturing is carried out at pH 4 to 9, preferably at around pH 7 to 25° to 35°C, preferably 28 to 33°C for about 5 to 20 days, preferably about 5 to 11 days. However, it is to be understood that such culture conditions as medium composition, pH, temperature and agitation may suitably be varied to obtain the optimum result according to the kind of strain used and external conditions. After culturing, the culture solution is subjected to, for example, a centrifugation to separate the cells from the solution. The antibiotic 76—11 thus produced can be isolated from the culture medium by any conventional method that is usually used for the isolation of metabolite. For example, a method using solubility difference between the antibiotic 76—11 and impurity, a method using difference of adsorptive affinity between them, or a combination thereof may be used and repeated, if needed. Specifically, the antibiotic 76—11 produced exists both in the culture solution and within the cells, and can be extracted from the culture solution, using ethyl or butyl acetates, chloroform, butanol, or the like, according to a difference in solubility between them. The cells are extracted with water-containing acetone or water-containing methanol, the organic solvents are evaporated under reduced pressure, and the aqueous solution thus obtained is extracted with ethyl acetate or the like. Both the extracted solutions are combined and concentrated to give a crude extract of the antibiotic 76—11. As the crude extract contains a great amount of impurity, it is subjected to adsorption chromatography using silica gel, alumina or the like and then purified. For example, the crude extract dissolved in a small amount of benzene is introduced in a silica gel column previously conditioned with benzene. Then, elution is conducted first with benzene, then with a mixture of benzene and ethyl acetate in which the thyl acetate content is gradually increased. The eluate is fractionated using a fraction collect r. The fractions showing a biological activity are collected and concentrated to give a purified powder. For further purification, the similar procedure f silica gel

chromatography is repeated. Still further purification is carried ut by preparative thin layer chromat graphy, if n cessary. C ncentrati n und r reduced pressure gives a purified product which is then dissolved in a small amount of methan l and is refrigerated, and the antibiotic 76—11 is separated ut as a colorless crystal. The crystal is separat d by filtration and dried to give pur antibi tic 76—11.

Th antibiotic 76—11 thus obtained xhibits th foll wing physic chemical and biol gical prop rties.

Physicochemical and biological properties of the antibiotic 76—11

(1) Elemental Analysis:
Free acid: C; 62.61%, H; 8.27%, N; 0%
Na salt: C; 60.57%, H; 8.04%, N; 0%

(2) Molecular Weight:
843 (measured by the titration method)
873 (measured by the FD mass spectrum method)

(3) Melting Point:
Free acid: 108—112°C
Na salt: 210—212°C (Decomposed)

(4) Specific Rotatory Power:
$[\alpha]_D^{25}$+36.6° (C=0.382, in chloroform solution)

(5) Ultraviolet Absorption Spectrum:
The maximum absorption bands;
In MeOH and HCl—MeOH:

λmax=217 mμ ($E_{1cm}^{1\%}$ 303)
262 mμ ($E_{1cm}^{1\%}$ 182)
301 mμ ($E_{1cm}^{1\%}$ 68)

In alkaline MeOH:

λmax=260 mμ ($E_{1cm}^{1\%}$ 87)
308 mμ ($E_{1cm}^{1\%}$ 50)

(6) Infrared Absorption Spectrum:
Main specific absorption bands in KBr plate:

Free acid: 3450, 2960, 1720, 1640, 1610, 1578, 1446, 1380, 1315, 1292, 1250, 1209, 1151, 1100, 1035, 975, 940 cm^{-1}
Na salt: 3390, 2960, 1718, 1640, 1609, 1578, 1450, 1380, 1340, 1316, 1250, 1197, 1152, 1108, 1092, 1060, 1040, 1002, 980, 930 cm^{-1}

(7) Solvent solubility:
Easily soluble in benzene, chloroform, ethyl acetate and acetone, soluble in methanol, ethanol and dimethylformamide, and hardly soluble in water and hexane.

(8) Coloring reactions:
Positive to potassium permanganate reaction but negative to periodic acid-benzidine reaction.

(9) Basicity, acidity or neutrality:
Acidic substance, pKa' 4.6 (in 66.7% dioxane).

(10) Color:
Colorless crystal.

(11) Antimicrobial activity:
The minimum concentration for inhibiting growth of various microorganisms on bouillon agar culture medium is as shown below.

Microorganisms tested	The minimum concentration for inhibiting growth (mcg/ml)
Staphylococcus aureus 209 P	0.4
Staphylococcus aureus (multi resistant)	0.4
Bacillus subtilis PC 1219	0.4
Bacillus subtilis H 17	0.4
Bacillus subtilis M 45 (rec$^-$)	0.4
Mycobacterium SP 607	0.4
Mycobacterium phlei	0.4
Mycobacterium avium	0.4
Escherichia coli	>100
Salmonella typhimurium	>100

(12) Action against tumor cell:
The antibiotic 76—11 shows an induction of differentiation against Friend leukemia and Myeloid leukemia cells in concentration of 1 to 100 mcg/ml.

(13) Toxicity for mice:
Abdominal administration of 50, 100 and 200 mg/kg of the antibiotic 76—11 in the form of CMC suspension showed no toxicity for mice.

Comparing the physicochemical and biological properties of the antibiotic 76—11 described above with those of known antibiotics, the antibiotic 76—11 seems to belong classified in the so-called polyether ionophore antibiotic group in that it is an acidic substance and the Na salt thereof is soluble in oil; that it shows a strong activity against gram-positive bacteria and acid-fast bacteria; that it does not contain nitrogen in the molecular structure and the like. However, no substances show the same physicochemical properties, especially the specific ultraviolet absorption spectrum bands of the antibiotic 76—11 as described above and, therefore, the antibiotic 76—11 was concluded to be a new antibiotic and was named accordingly.

Next, the anticoccidiosis agent of the present invention will be described. When using the antibiotic 76—11 as an anticoccidiosis agent, said antibiotic 76—11 may be administered as such or in the form of a feed additive. Examples of feed materials include barley flour, wheat flour, rye flour, corn flour, soybean flour, soybean cake, cole seed cake, rice bran, exoleated bran, white potato powder, sweet potato powder, the other various starches, bean-curds (tōfu) bran, yeast, fish meal, fermentation residue, and the like. The antibiotic 76—11 may also be added to some conventional feed additive such as various vitamins, minerals, preservatives, enzyme preparations, proteins, carbohydrates, amino acids, febrifuges, sedative agents, antiphlogistics and microbiocides.

The content of the effective ingredient varies according to the type and stage of the disease, the age of the domestic fowls and generally is in the range of about 5 to 200 ppm, preferably 10 to 100 ppm.

The present invention provides an excellent anticoccidiosis agent which is extremely effective against coccidiosis of domestic fowls and shows no toxicity and no side effects.

Next, a growth accelerating and feed efficiency increasing agent of the present invention for domestic animals and fowls will be described.

The growth accelerating and feed efficiency increasing agent of this invention may be prepared by adding the antibiotic 76—11 to feed or to the drinking water of domestic animals as such or in the form of a dispersion or solution in diluent, although the agent may also be used in the form of a powder, tablet, capsule, granule or pill which may be prepared by mixing the antibiotic 76—11 with or without physiologically harmless s lid or liquid dilu nt. For addition t feed, a premix previously prepared is preferably used. The premix may be prepared by mixing the purified or crude products or the cells containing the effective ingredient with physi logically acceptable, solid or liquid carrier. Examples of solid carriers include wh at flour, s ybean flour, rice bran, corn fl ur, starch, glucos , yeast, fish meal, talc,

diatomaceous earth, etc. and examples of liquid carriers include physiol gic saline, distilled water, physi logically acceptable organic solv nts, etc.

Other appropriate auxiliaries or additives such as emulsifying, dispersing, suspending, wetting, concentrating or gelatinizing agents, microbicid s, preservatives, enzym preparations, antibiotics, or lact bacilli formulations may be mixed with the agent of this invention.

The content of the effective ingredient in the premix may appropriately be varied according to the kind of domestic animals or fowls.

The concentration of the antibiotic 76—11 in the growth accelerating and feed efficiency increasing agent of the present invention may be modified according to the kind and age of the fowls, and so on. For example, domestic fowls such as chicken, quail, turkey, guinea fowl, duck, goose, etc. are administered a feed that contains the antibiotic 76—11 in the amount of 5 to 200 ppm, preferably 10 to 100 ppm. Feed containing the effective ingredient may be used in the amount of 5 to 200 ppm, preferably 10 to 100 ppm for pigs, rabbits, etc. and 1 to 100 ppm, preferably 2 to 50 ppm for ruminants such as cattle, sheep, goats, etc.

The antibiotic 76—11 of this invention has a low toxicity and the advantage that the administration of the antibiotic 76—11 combined with the other antibiotic to domestic animals or fowls shows substantially no side effects. The administration of polyether antibiotics such as Salino-mycin and Monencin to domestic animals or fowls in a usual dosage, combined with triacetyl Oleandmycin or Pleuromutilin fumarate in a usual dosage generally brings about temporary anorexia or inappetence, hindleg paralysis and the like and therefore, care should formerly have been taken to avoid administration of the polyether antibiotic simultaneously with or close to the administration of the latter substances. Conversely the antibiotic 76—11 presents no such dangers but is an excellent agent which is capable of achieving both the desired growth acceleration and increased feed efficiency of domestic animals and fowls.

The administration of the agent of this invention to domestic animals and fowls accelerates the formation of propionic acid in the digestive organs thereof and inhibits the increase in viscosity of rumen liquid, which results not only in the prevention of dysentery, bloat and ketosis of the animals, but also in the acceleration of healthy growth of the animals and fowls and increased feed efficiency.

In the anticoccidiosis agent and the growth accelerating and feed efficiency increasing agent of the present invention, the effective ingredient or the antibiotic 76—11 may be used in the form of the following: the purified or crude products; the cells containing the antibiotic 76—11; a physiologically acceptable metal salt (such as sodium, calcium, etc.), an organic acid ester (such as propionic acid ester, valeric acid ester, etc), or a metal complex (such as a zinc complex).

The present invention will now be described in detail with reference to the following Examples and Test Examples, which do not limit the present invention.

Except where specified, "%" and "parts" respectively mean "% by weight" and "parts by weight" in the specification and claims.

The following Example shows a process for producing the antibiotic 76—11.

Example 1

Production of the antibiotic 76—11

The above-mentioned Sp. 76—11 (FERM BP-83) previously cultured in the slant culture was inoculated in a culture medium comprising 3% oatmeal, 1.5% glycerin and 0.5% meat extract (pH: around 6.5) and cultured at 28°C for 11 days with shaking. Every 3 ml of the culture solution was inoculated into a fresh culture medium containing the same constituents and cultured for a further 7 days.

140 ml of the culture solution thus obtained was inoculated into 18 l of a culture medium comprising the same constituents in a jar fermenter and cultivated at 28°C for 210 hours with aeration at 18 l/min and agitation at 330 rpm.

After culturing, 400 g of diatomaceous earth (Radiolite 700) was added to the culture solution and then the solution was subjected to a centrifugal filtration. The supernatant obtained (14 l, pH 7.8) was extracted twice with 8 l and 5 l of ethylacetate respectively. Alternatively the cells were extracted twice with 9 l and 6 l of acetone respectively. The extracted solution was concentrated in vacuo to give 2 l of an aqueous solution (pH 8) which was then extracted twice with each 1 l of ethylacetate. The latter ethylacetate extracted solution was combined with the former one from the supernatant and concentrated in vacuo. The residue thus obtained was dissolved in a small amount of benzene, which was introduced into a silica gel column (φ3 cm×50 cm) previously conditioned with benzene. Elution was carried out with 2 l of benzene, in turn, 1 l of benzene-ethylacetate (5:l (v/v)), 1 l of benzene-ethylacetate (1:1 (v/v)). Active fractions were eluted with benzene-ethylacetate (1:1 (v/v)), collected, concentrated in vacuo and treated with methanol to give about one gram of crude crystals. Several recrystallizations from methanol gave 400 mg pure crystals of sodium salt of the antibiotic 76—11.

Next, a test Example of the anticoccidiosis agent of this invention is described.

Test Example 1

Anticoccidiosis agent

Agents tested:

The effective ingredients used in this test were uniformly blended to obtain a giv n conc ntration with a anticoccidiosis agent-free perfect combination feed for chicks (produced by Oriental Yeast Co.;

formulation shown in Table 1). The feeds thus btain d w re freely taken by chickens fr m 2 days before infection with oocyst to the end of the test (8 days after the infecti n). Salinomycin was used as the control.

TABLE 1

Corn	61.2 %
Wheat flour	5.13%
Soybean oil	3.07%
Soybean meal	15.4 %
Fish meal	10.3 %
Lucerne meal	3.07%
Calcium carbonate	0.3 %
Salt (NaCl)	0.5 %
Vitamine mix	1.03%

Chickens used:

The chickens used in this test were healthy cocks of egg-laying fowl (Shaver Starcross) which were 7 days old (9 days old when infected) and had been bred under perfect conditions of prevention of coccidiosis infection. Each group comprised five chickens.

Oocyst inoculated and quantity of inoculation:

The oocyst used for the infection was a sensitive strain of *Eimeria tenella*. Every chicken was inoculated orally through the crop with fully grown oocysts (5×10^4) using a metal sonde.

Judgment of effect:

The effect of the agents was determined by the anti-coccidiosis index (ACI) which was calculated by the following formula:

ACI=(Relative Increase in Weight+Survival Rate)−(Oocyst Value+Disease Value).

(i) Relative increase in weight

The weight of the chickens tested was measured at the start of the test (2 days before the inoculation or −2 day), the inoculation (0 day), 2, 4, 5, 6, 7 and 8 days after inoculation. At the end of the test, the increase in weight of each test group was measured and the relative increase in weight was calculated based on weight of the control group (100) which was bred with the anticoccidiosis agent-free feed and not inoculated.

(ii) Oocyst value

The number of oocysts in the caecum was counted 8 days after the inoculation by homogenizing the intestinal canal. The oocyst value was defined as follows:

Number of oocysts found in the intestinal canal	Oocyst value
$0.0-0.1\times10^6$	0
$0.1-1.0\times10^6$	1
$1.0-5.0\times10^6$	10
$5.0-11.0\times10^6$	20
$>11.0\times10^6$	40

(iii) Disease index f the intestinal canal

The chickens tested were anatomized at the end of the test (8 days after the inoculation) and the intestinal canal was examined with the naked dye to determine the disease index. The disease index was defined as follows and the disease value was defined as ten times the value of the disease index.

0, (−) .... The caecum is quite normal. If bleeding spot is found, (−) is changed to (+).
1, (+) .... The caecum is n rmal in shape. The c ntent therein is slightly fluid and y ll wish. Slight swelling is found partly on a mucous membran f th caecum which becomes whitish.
2, (++) .... The caecum is g n rally normal in shape. Swelling is f und on the whol surface of a mucous membrane. No bleeding is found in the sample. Mucus is slightly yellowish and faded. A few white spot-like necroses or bleeding spots are found in a mucous membrane.
3, (+++) .... The caecum is clearly withered and altered in shape, and is a little longer than the rectum. The content is quite abnormal and is often filled with coagulated blood or white-gray, cheese-like degenerated matter. The wall of the caecum is clearly swollen and easily broken and sometimes bleeding spots still remain. The disease reaches the base of the caecum but not the rectum.
4, (++++) .... Withering and deformation of the caecum are very noticeable. The caecum is sausage-like in shape and is not longer than the rectum. The disease extends to almost one third or fourth of the rectum. The other points are the same as those described in item (3).'

(iv) Feed demand

The feed demand of each group tested was calculated from the average increase in weight and the total amount of feed ingested from the start to the end of the test (10 days).

$$\text{Feed demand} = \frac{\text{Amount of feed ingested}}{\text{Increase in weight}}$$

The results are given in Tables 2 and 3.

TABLE 2

Group		Amount of feed ingested (g)	Increase in weight (g)	Feed demand index
Control: No infection No administration		177.2	96.4	1.84
Control: Infection No administration		177.4	87.0	2.04
The antibiotic 76—11	50 ppm	179.3	90.4	1.98
The antibiotic 76—11	100 ppm	174.4	80.4	2.17
Salinomycin	50 ppm	170.6	91.8	1.86

TABLE 3

Group		Control no infection no d sage	Contr l infecti n[1] no dosage	The antibiotic 76—11 50 ppm	100 ppm	Salinomycin 50 ppm
Change in weight (g)	days −2	63.6 ±1.56	63.0 ±1.18	63.4 ±0.98	63.4 ±1.78	63.6 ±1.08
	0	81.6 ±3.12	79.6 ±2.14	78.0 ±2.98	79.6 ±1.91	78.4 ±1.21
	2	100.8 4.42	100.6 +2.96	96.0 ±4.00	96.0 ±2.77	98.8 ±1.52
	4	119.0 ±4.76	122.2 ±4.99	113.8 ±4.99	113.4 ±3.11	118.8 ±1.96
	5	129.4 ±5.20	125.6 ±4.82	120.5 ±6.41	121.6 ±4.31	127.8 ±2.65
	6	137.4 ±5.14	133.4 ±5.22	131.5 ±6.33	127.4 ±4.61	145.8 ±2.96
	7	147.2 ±5.40	141.6 ±6.24	143.0 ±7.29	136.2 ±5.06	145.8 ±4.00
	8	160.0 ±5.74	150.0 ±6.14	153.8 ±7.76	143.8 ±6.28	155.4 ±4.74
Increase in weight		78.4	70.4	75.8	64.2	77.0
Relative increase in weight		100	89.8	96.7	81.9	98.2

TABLE 3 (continued)

	4	–	+++	+++	–	+
Bloody excrement[3] and death[4]	5	–	++	+	–	++
	6	–	++	+	–	+
	7	–	+	–	–	–
	8	–	–	–	–	–
Survival		100	100	80	100	100
The number of oocyst found in the intestinal canal		0	$2{,}381\times10^7$	8.86×10^6	5.52×10^5	6.91×10^6
Oocyst value		0	40	20	1	20
Disease in the intestinal canal	++++	0	3	1	0	1
	+++	0	2	1	0	1
	++	0	0	3	0	0
	+	0	0	0	4	3
	–	5	0	0	1	0
Disease value		0	36	26	8	20
Anticoccidiosis index		200	114	131	173	158

[1] Infection: 5×10^4 Oocysts of Eimeria tenella/one chicken
[2] ±: Standard error
[3] Degree of bloody excrement: –; no +; less than 10% ++; 10—30% +++; 30—50% ++++; more than 50%
[4] Numerical values in parentheses show the number of dead chicken.

Thus, the administration of anticoccidiosis agent of the present invention shows considerable improvements as regards bloody excrement, disease of the caecum and the number of oocysts detected in the intestinal canal as compared with the control group which was infected but not administered with said agent.

Further, it was found that the chickens to which the anticoccidiosis agent of the present invention was administered showed no symptoms of coccidiosis even after being infected, i.e. the growth of oocysts of *Eimeria tenella* was substantially inhibited and, accordingly, the antibiotic 76—11 of the present invention proved itself extremely useful as an anticoccidiosis agent.

The growth accelerating and feed efficiency increasing agent of the present invention will now be described with reference to the following Formulating and Test Examples.

Formulating Example 1

The antibiotic 76—11	1%
Corn starch	99%

Both substances were pulverized and uniformly blended to give a premix containing 1% of the antibiotic 76—11.

Formulating Example 2

The antibiotic 76—11 (crude, purity 40%)	2.5%
Wheat bran	97.5%

Both substances were pulverized and uniformly blended to give a premix containing 1% of the antibiotic 76—11.

Formulating Example 3

1000 grams of dry cells containing 0.96% of the antibiotic 76—11 is pulverized and is ready for use as a premix.

Test Example 2

Sixty cockerels (day-old) for broiling (Shaver Star Brow) were classified into three groups so that each group had the same average weight. Feeds to which the premix prepared according to Formulating Example 1 was added to contain the antibiotic 76—11 in the concentration of 25 ppm and 50 ppm, were fed continuously for seven weeks to the first and second groups respectively, and a feed to which the premix was not added was fed to the third group. The increase in weight and the total amount of feed ingested during this period were measured to calculate the feed demand index of each group. The results are shown in Table 4.

A feed for the former half was used from the beginning of the test to 21 days afterwards a feed for the latter half was used, the feeds for both the former and the latter were those for broilers and contained no antibiotic, and the nutrient contents of each of the feeds were as follows:

	For former half use	For latter half use
Crude protein	23.4%	20.4%
Crude fat	5.5%	6.1%
Crude fiber	3.9%	2.8%
Crude ash	5.3%	5.5%
Metabolizable energy	3,020 Cal	3,100 Cal

TABLE 4

Group	Additive	Weight (g) At the start	Weight (g) At the end	Increase in weight (g)	Feed ingested (g)	Feed demand index
1.	The antibiotic 76—11 25 ppm	47 (100)	1861 (105.9)	1814 (106.1)	3140 (99.8)	1.731 (94.1)
2.	The antibiotic 76—11 50 ppm	47 (100)	1879 (106.1)	1832 (107.1)	3120 (99.2)	1.703 (92.6)
3.	Control (No. addition)	47 (100)	1757 (100)	1710 (100)	3145 (100)	1.839 (100)

Note:

Numerical values in parentheses show the proportion (%) to Control.

$$\text{Feed demand index} = \frac{\text{Amount of feed ingested}}{\text{Increase in weight}}$$

As seen in the results described above, the administration of the antibiotic 76—11 in the concentration of 25 ppm and 50 ppm accelerated growth by 6.1% and 7.1% respectively, and increased the feed efficiency by 5.9% and 7.4% respectively.

It has been found that administration of the antibiotic 76—11 to herbivorous animals increases the ratio of propionic acid to acetic and butyric acids, which are formed within the rumen of ruminants such as cattle, sheep and goats, or within the large intestine of animals having a single stomach such as rabbits and pigs. On the other hand it is known that propionic acid is superior to acetic or butyric acids in utilization of volatile fatty acids as energy. Accordingly, it is believed that the administration of the antibiotic 76—11 accelerates the formation of propionic acid in a digestive organ, which accelerates growth of the animals and increases the feed efficiency. In addition, the administration of the antibiotic 76—11 can not only prevent the bloat or ketosis which are frequently found in beef cattle to which a large amount of concentrated feed is fed, but also cure animals already suffering from such diseases. As an example of the

applicati ns f r ruminants, the following ne is describ d, wh rein a feed to which the antibiotic 76—11 is uniformly added and blended is fed to a calf.

Test Example 3

Four castrated, Holstein calves w ighing about 330 kg (nine month ld) were divided into two groups. A feed to which the antibiotic 76—11 was added in a concentration of 30 ppm was fed to the first group, while a feed to which the antibiotic 76—11 had not been added was fed to the second group, respectively for 16 weeks continuously. The increase in weight and the total amount of feed ingested during this period were measured to calculate the feed demand index of each group. The results are shown in Table 5. The viscosity of and the content of volatile fatty acid in the rumen liquid collected by a catheter via the nose just before and after the test was measured to calculate the molar ratio of propionic acid to acetic acid contained therein. The results are shown in Table 6.

A feed for beef cattle "Kumiai New King Beef for the latter half" formulated by Zen-nō, Japan, which is freely ingested, and a dried rice plant (3 kg/day per calf) were fed to the animals. The nutrient contents of "Kumiai New King Beef for the latter half" are as follows:

Crude protein	11.5%
Crude fat	2.0%
Crude fiber	9.0%
Crude ash	9.0%
Digestible crude protein	9.0%
Total digestible nutrient	72.0%

As seen from Table 5, the administration of the antibiotic 76—11 in the concentration of 30 ppm lowered the feed demand index by 15%.

TABLE 5

Group	Additive	Weight (kg) At the start	Weight (kg) At the end	Increase in weight (kg)	Feed ingested (kg)	Feed demand index
1.	The antibiotic 76—11 30 ppm	332 (99)	457 (101)	125 (108)	880 (91)	7.04 (85)
2.	Control (No addition)	335 (100)	451 (100)	116 (100)	965 (100)	8.32 (100)

Note:
Numerical values in parentheses show the proportion (%) to Control.

TABLE 6

Group	Additive	Before the test: Viscosity (mPa · s)	Before the test: Propionic acid/acetic acid	After the test: Viscosity (mPa · s)	After the test: Propionic acid/acetic acid
1.	The antibiotic 76—11 30 ppm	4.1 (114)	0.54 (98)	3.2 (43)	0.81 (180)
2.	Control	3.6 (100)	0.55 (100)	7.5 (100)	0.45 (100)

Not :
Numerical values in parentheses show the proportion (%) to C ntrol.

As s en from Table 6, the administration of the antibiotic 76—11 in the concentration of 30 ppm inhibits the increase in the viscosity f the rumen liquid, which will be effective in preventing bloat. Furth r, the

antibiotic 76—11 is believed to be effective in preventing ketosis, since the administration thereof increases the ratio of propionic acid produced.

The following shows, as an application to a herbivorous animal having a single stomach, an example in which a feed to which the antibiotic 76—11 is uniformly added and blended is fed to pigs.

Test Example 4

Ten landrace pigs (two months old) that are full brothers were classified into two groups of five each so that each group had the same average weight and sex ratio. A feed to which the antibiotic 76—11 was added in a concentration of 50 ppm was fed to the first group, while a feed to which the antibiotic 76—11 was not added, was fed to the second group, respectively for ten weeks continuously. The increase in weight and the total amount of feed ingested during this period were measured to calculate the feed demand index of each group. The results are shown in Table 7. In addition, the content of volatile fatty acid (VFA) in the excrement was measured at the end of the test. The results are given in Table 8.

A feed for a pig containing no antibiotic was used with the following formulation:

Cereals (corn, milo, wheat)	78%
Soybean oil cake	13%
Fish meal	5%
Others (Sodium chloride, Calcium carbonate, Calcium phosphate, etc.)	4%

TABLE 7

Group	Additive	Weight (kg) At the start	Weight (kg) At the end	Increase in weight (kg)	Feed ingested (kg)	Feed demand index
1.	The antibiotic 76—11 50 ppm	14.07 (100)	53.18 (112)	39.11 (117)	96.37 (106)	2.464 (91)
2.	Control (No. addition)	14.08 (100)	47.53 (100)	33.45 (100)	90.75 (100)	2.713 (100)

Note:
Numerical values in parentheses show the proportion (%) to Control.

As seen from Table 7, the administration of the antibiotic 76—11 in the concentration of 50 ppm accelerated growth by 17% and lowered the feed demand index by 9%.

TABLE 8

Group	Additive	Total amount of VFA (mmol/g)	Molar ratios of each fatty acid to the total amount of VFA: Propionic acid	Acetic acid	Butyric acid
1.	The antibiotic 76—11 50 ppm	0.176 (96)	32.7 (11.8)	39.9 (96)	15.3 (84)
2.	Control	0.183 (100)	27.8 (100)	41.7 (100)	18.3 (100)

Note:
Numerical values in parentheses show the proportion (%) to Control.

It is known that the amount of volatile fatty acid (VFA) absorbed by the intestinal canal of a pig does not change according to the kind of fatty acid. Accordingly, it is believed that the increase in the content of propionic acid contained in excrement, by the administration of the antibiotic 76—11 in the concentration of 50 ppm, reflects the increase in the production of propionic acid in the intestinal canal.

Claims f r the Contracting States BE CH DE FR GB IT LI LU NL SE

1. The micr organism Actinomadura Sp. 76—11, FERM BP-83.

2. Th antibiotic 76—11 obtained by culturing the micro rganism FERM-BP 83, and isolating the antibiotic 76—11 from the culture.

3. The antibiotic 76—11, characterized by the following physicochemical and biological properties:

(1) Elemental analysis:
Free acid: C; 62.61%, H; 8.27%, N; 0%
Na salt: C; 60.57%, H; 8.04%, N; 0%

(2) Molecular weight:
843 (measured by the titration method)
873 (measured by the FD mass spectrum method)

(3) Melting point:
Free acid: 108—112°C
Na salt: 210—212°C (Decomposed)

(4) Specific rotatory power:
$[\alpha]_D^{25}$ +36.6° (C=0.382, in chloroform solution)

(5) Ultraviolet absorption spectrum:
The maximum absorption bands;
In MeOH and HCl—MeOH:

λmax=217 mμ ($E_{1cm}^{1\%}$ 303)
262 mμ ($E_{1cm}^{1\%}$ 182)
301 mμ ($E_{1cm}^{1\%}$ 68)

In alkaline MeOH:

λmax=260 mμ ($E_{1cm}^{1\%}$ 87)
308 mμ ($E_{1cm}^{1\%}$ 50)

(6) Infrared absorption spectrum:
Main specific absorption bands in KBr plate:

Free acid: 3450, 2960, 1720, 1640, 1610, 1578, 1446, 1380, 1315, 1292, 1250, 1209, 1151, 1100, 1035, 975, 940 cm^{-1}
Na salt: 3390, 2960, 1718, 1640, 1609, 1578, 1450, 1380, 1340, 1316, 1250, 1197, 1152, 1108, 1092, 1060, 1040, 1002, 980, 930 cm^{-1}

(7) Solvent solubility:
Easily soluble in benzene, chloroform, ethyl acetate and acetone, soluble in methanol, ethanol and dimethylformamide, and hardly soluble in water and hexane

(8) Coloring reactions:
Positive to potassium permanganate reaction but negative to periodic acid—benzidine reaction

(9) Basicity, acidity or neutrality:
Acidic substance, kPa' 4.6 (in 66.7% dioxane)

(10) Color:
Colorless crystal

(11) Antimicrobial activity:
Growth inhibition against gram positive coccus, bacillus and acid-fast bacteria revealed in the concentration of 0.4 mcg/ml.

4. A process for producing the antibiotic 76—11 defined in claims 2 and 3 which comprises culturing an antibiotic 76—11-producing micr organism belonging to the genus *Actinomadura* and is lating the antibiotic 76—11 from the culture products.

5. The process of claim 4, wher in the antibiotic 76—11-producing microorganism belonging to the genus *Actinomadura* is FERM-BP 83.

6. The process of claims 4 and 5, wherein the isolation is carried out by collecting a cell free extract

fr m the culture medium and fracti nating the extract by a chr matographic method to obtain the antibi tic 76—11.

7. An antic ccidiosis agent comprising the antibiotic 76—11 defined in claims 2 and 3 as an effective ingredient.

8. A growth accelerating and feed efficiency increasing agent f r domestic animals and fowls comprising the antibiotic 76—11 defined in claims 2 and 3 as an effective ingredient.

9. A method for accelerating the growth of domestic animals and, fowls and increasing the feed efficiency thereof which comprises administering an effective amount of the antibiotic 76—11 defined in claims 2 and 3 to the animals and fowls.

10. A feed for domestic fowls comprising the antibiotic 76—11 defined in claims 2 and 3 in the amount effective against coccidiosis of the fowls.

11. The feed of claim 10, wherein the antibiotic 76—11 is contained in the concentration of about 5 to 200 ppm.

12. A feed for domestic animals or fowls comprising the antibiotic 76—11 defined in claims 2 and 3 in the amount effective in accelerating the growth of the animals or fowls and the increasing feed efficiency.

13. The feed of claim 12, wherein the antibiotic 76—11 is contained in a concentration of about 1 to 200 ppm.

Claims for the Contracting State AT

1. A process for obtaining the antibiotic 76—11, having the following physicochemical and biological properties:

(1) Elemental analysis:
Free acid: C; 62.61%, H; 8.27%, N; 0%
Na salt: C; 60.57%, H; 8.04%, N; 0%

(2) Molecular weight:
843 (measured by the titration method)
873 (measured by the FD mass spectrum method)

(3) Melting point
Free acid: 108—112°C
Na salt: 210—212°C (Decomposed)

(4) Specific rotatory power:
$[\alpha]_D^{25}$+36.6° (C=0.382, in chloroform solution)

(5) Ultraviolet absorption spectrum:
The maximum absorption bands;
In MeOH and HCl-MeOH:

λmax=217 mμ ($E_{1cm}^{1\%}$ 303)
262 mμ ($E_{1cm}^{1\%}$ 182)
301 mμ ($E_{1cm}^{1\%}$ 68)

In alkaline MeOH:

λmax=260 mμ ($E_{1cm}^{1\%}$ 87)
308 mμ ($E_{1cm}^{1\%}$ 50)

(6) Infrared absorption spectrum:
Main specific absorption bands in KBr plate:

Free acid: 3450, 2960, 1720, 1640, 1610, 1578, 1446, 1380, 1315, 1292, 1250, 1209, 1151, 1100, 1035, 975, 940 cm^{-1}
Na salt: 3390, 2960, 1718, 1640, 1609, 1578, 1450, 1380, 1340, 1316, 1250, 1197, 1152, 1108, 1092, 1060, 1040, 1002, 980, 930 cm^{-1}

(7) Solvent solubility:
Easily soluble in benzene, chloroform, ethyl acetate and acetone, soluble in methanol, ethanol and dimethylformamide, and hardly soluble in water and hexane

(8) Coloring reactions:
Positive to potassium permanganate reaction but negative to periodic acid—benzidine reaction

(9) Basicity, acidity or neutrality:
Acidic substance, pKa' 4.6 (in 66.7% dioxane)

(10) Color:
Colorless crystal

(11) Antimicrobial acitivity:
Growth inhibition against gram positive coccus, bacillus and acid-fast bacteria revealed in the concentration of 0.4 mcg/ml, characterized by culturing an antibiotic 76—11-producing microorganism belonging to the genus *Actinomadura* and isolating the antibiotic 76—11 from the culture products.

2. The process of claim 1, wherein the antibiotic 76—11-producing microorganism belonging to the genus *Actinomadura* is FERM-BP 83.

3. The process of claims 1 and 2, wherein the isolation is carried out by collecting a cell free extract from the culture medium and fractionating the extract by a chromatographic method to obtain the antibiotic 76—11.

4. A process for preparing an anticoccidiosis agent characterized by combining the antibiotic 76—11 defined in claim 1 as an effective ingredient with usual carriers and additives.

5. A process for preparing a growth accelerating and feed efficiency increasing agent for domestic animals and fowls characterized by combining the antibiotic 76—11 defined in claim 1 as an effective ingredient with usual carriers and additives.

6. A method for accelerating the growth of domestic animals and fowls and increasing the feed efficiency thereof which comprises administering an effective amount of the antibiotic 76—11 defined in claim 1 to the animals and fowls.

7. A process for preparing a feed for domestic fowls characterized by combining the antibiotic 76—11 defined in claim 1 in the amount effective against coccidiosis of the fowls with the other usual feed ingredients.

8. The process of claim 7, wherein the antibiotic 76—11 is used in the concentration of about 5 to 200 ppm.

9. A process for preparing a feed for domestic animals or fowls characterized by combining the antibiotic 76—11 defined in claim 1 in the amount effective in accelerating the growth of the animals or fowls and in increasing feed efficiency with the other usual feed ingredients.

10. The process of claim 9, wherein the antibiotic 76—11 is used in a concentration of about 1 to 200 ppm.

Patentansprüche für die Vertragsstaaten BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Der Mikroorganismus Actinomadura Sp. 76—11, FERM BP-83.

2. Antibiotikum 76—11, erhalten durch Kultivieren des Mikroorganismus FERM-BP 83 und Abtrennen des Antibiotikums 76—11 aus der Kultur.

3. Antibiotikum 76—11, gekennzeichnet durch die folgenden physikochemischen und biologischen Eigenschaften:

(1) Elementaranalyse:
Freie Säure: C: 62,61%, H: 8,27%; N: 0%
Natriumsalz: C: 60,57%; H: 8,04%; N: 0%

(2) Molekulargewicht:
843 (gemessen nach dem Titrationsverfahren)
873 (gemessen nach dem FD-Massenspektrum-Verfahren).

(3) Schmelzpunkt:
Freie Säure: 108 bis 112°C
Natriumsalz: 210 bis 212°C (Zers.).

(4) Spezifisches Drehvermögen:
$[\alpha]_D^{25}$+36,6° (C=0,382; in Chloroformlösung).

(5) UV-Absorptionsspektrum:
Maximale Absorptionsbanden
In MeOH und HCl-MeOH:

λmax=217 mμ ($E_{1cm}^{1\%}$ 303)
262 mμ ($E_{1cm}^{1\%}$ 182)
301 mμ ($E_{1cm}^{1\%}$ 68)

In alkalischem MeOH:

λmax=260 mμ ($E^{1\%}_{1cm}$ 87)
308 mμ ($E^{1\%}_{1cm}$ 50)

(6) IR-Absorptionsspektrum:
Stärkste spezifische Absorptionsbanden im KBr-Plättchen:

Freie Säure: 3450, 2960, 1720, 1640, 1610, 1578, 1446, 1380, 1315, 1292, 1250, 1209, 1151, 1100, 1035, 975, 940 cm^{-1}
Na-Salz: 3390, 2960, 1718, 1640, 1609, 1578, 1450, 1380, 1340, 1316, 1250, 1197, 1152, 1108, 1092, 1060, 1040, 1002, 980, 930 cm^{-1}.

(7) Löslichkeit in Lösungsmitteln:
Leicht löslich in Benzol, Chloroform, Äthylacetat und Aceton, löslich in Methanol, Äthanol und Dimethylformamid und kaum löslich in Wasser und Hexan.

(8) Farbreaktionen:
Positive Kaliumpermanganat-Reaktion, jedoch negative Perjodsäure-Benzidin-Reaktion.

(9) Basizität, Azidität oder Neutralität:
Saurer, Stoff; pKa' 4,6 (in 66,7% Dioxan).

(10) Farbe:
Farblose Kristalle.

(11) Antimikrobielle Wirksamkeit:
Wachstumshemmung gegen gram-positive Kokken, Bazillen und säurefeste Bakterien in der Konzentration von 0,4 mcg/ml.

4. Verfahren zur Herstellung des Antibiotikum 76—11 gemäß Ansprüche 2 und 3, dadurch gekennzeichnet, daß man einen zur Art *Actinomadura* gehörenden, Antibiotikum 76—11 erzeugenden Mikroorganismus kultiviert und das Antibiotikum 76—11 aus den Kulturprodukten gewinnt.

5. Verfahren nach Anspruch 4, dadurch gekennzeichnet, daß der zur Art *Actinomadura* gehörende, Antibiotikum 76—11 produzierende Mikroorganismus FERM-BP 83 ist.

6. Verfahren nach den Ansprüchen 4 und 5, dadurch gekennzeichnet, daß zur Gewinnung von Antibiotikum 76—11 ein zellfreier Extrakt aus dem Kulturmedium gesammelt und durch Chromatographie fraktioniert wird.

7. Antikokzidiosemittel, enthaltend Antibiotikum 76—11 gemäß Ansprüche 2 und 3 als Wirkstoff.

8. Das Wachstum beschleunigendes und die Futterverwertung erhöhendes Mittel für Haustiere und Geflügel, enthaltend Antibiotikum 76—11 gemäß Ansprüche 2 und 3 als Wirkstoff.

9. Verfahren zur Beschleunigung des Wachstums von Haustieren und Geflügel und zur Erhöhung ihrer Futterverwertung, dadurch gekennzeichnet, daß man den Tieren oder dem Geflügel eine wirksame Menge Antibiotikum 76—11 gemäß Ansprüche 2 und 3 verabreicht.

10. Futter für Hausgeflügel, enthaltend Antibiotikum 76—11 gemäß Ansprüche 2 und 3 in einer gegen Kokzidiose des Geflügels wirksamen Menge.

11. Futter nach Anspruch 10, dadurch gekennzeichnet, daß das Antibiotikum 76—11 in einer Konzentration von etwa 5 bis 200 T.p.M. enthalten ist.

12. Futter für Haustiere oder Geflügel, gekennzeichnet durch einen Gehalt an Antibiotikum 76—11 gemäß Ansprüche 2 und 3 in einer zur Beschleunigung des Wachstums der Tiere oder des Geflügels und zur Erhöhung ihrer Futterverwertung wirksamen Menge.

13. Futter nach Anspruch 12, dadurch gekennzeichnet, daß das Antibiotikum 76—11 in einer Konzentration von etwa 1 bis 200 T.p.M. enthalten ist.

Patentansprüche für den Vertragsstaat AT

1. Verfahren zur Gewinnung des Antibiotikums 76—11, mit den folgenden physikochemischen und biologischen Eigenschaften:

(1) Elementaranalyse:
Freie Säure: C: 62,61%; H: 8,27%; N: 0%
Natriumsalz: C: 60,57%; H: 8,04%; N: 0%

(2) Molekulargewicht:
843 (gemessen nach dem Titrationsverfahren)
873 (gemessen nach dem FD-Mass nspektrum-Verfahren).

(3) Schmelzpunkt:
Freie Säure: 108 bis 112°C
Natriumsalz: 210 bis 212°C (Zers.).

(4) Spezifisches Drehvermögen:
$[\alpha]_D^{25}$+36,6° (C=0,382; in Chloroformlösung).

(5) UV-Absorptionsspektrum:
Maximale Absorptionsbanden
In MeOH und HCl-MeOH:

λmax=217 mμ ($E_{1cm}^{1\%}$ 303)
262 mμ ($E_{1cm}^{1\%}$ 182)
301 mμ ($E_{1cm}^{1\%}$ 68)

In alkalischem MeOH:

λmax=260 mμ ($E_{1cm}^{1\%}$ 87)
308 mμ ($E_{1cm}^{1\%}$ 50)

(6) IR-Absorptionsspektrum:
Stärkste spezifische Absorptionsbanden im KBr-Plättchem:

Freie Säure: 3450, 2960, 1720, 1640, 1610, 1578, 1446, 1380, 1315, 1292, 1250, 1209, 1151, 1100, 1035, 975, 940 cm^{-1}
Na-Salz: 3390, 2960, 1718, 1640, 1609, 1578, 1450, 1380, 1340, 1316, 1250, 1197, 1152, 1108, 1092, 1060, 1040, 1002, 980, 930 cm^{-1}.

(7) Löslichkeit in Lösungsmitteln:
Leicht löslich in Benzol, Chloroform, Äthylacetat und Aceton, löslich in Methanol, Äthanol und Dimethylformamid und kaum löslich in Wasser und Hexan.

(8) Farbreaktionen:
Positive Kaliumpermanganat-Reaktion, jedoch negative Perjodsäure-Benzidin-Reaktion.

(9) Basizität, Azidität oder Neutralität:
Sauer, Stoff; pKa' 4,6 (in 66,7% Dioxan).

(10) Farbe:
Farblose Kristalle.

(11) Antimikrobielle Wirksamkeit:
Wachstumshemmung gegen gram-positive Kokken, Bazillen und säurefeste Bakterien in der Konzentration von 0,4 mcg/ml, dadurch gekennzeichnet, daß man einen zur Art *Actinomadura* gehörenden, Antibiotikum 76—11 erzeugenden Mikroorganismus kultiviert und das Antibiotikum 76—11 aus den Kulturprodukten gewinnt.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß der zur Art *Actinomadura* gehörende, Antibiotikum 76—11 produzierende Mikroorganismus FERM-BP 83 ist.

3. Verfahren nach den Ansprüchen 1 und 2, dadurch gekennzeichnet, daß zur Gewinnung von Antibiotikum 76—11 ein zellfreier Extrakt aus dem Kulturmedium gesammelt und durch Chromatographie fraktioniert wird.

4. Verfahren zur Herstellung eines Antikokzidiosemittels, gekennzeichnet durch Verbinden von Antibiotikum 76—11 gemäß Anspruch 1 als Wirkstoff mit üblichen Trägern und Zusatzstoffen.

5. Verfahren zur Herstellung eines das Wachstum beschleunigenden und die Futterverwertung erhöhenden Mittels für Haustiere und Geflügel, gekennzeichnet durch Verbinden von Antibiotikum 76—11 gemäß Anspruch 1 als Wirkstoff mit üblichen Trägern und Zusatzstoffen.

6. Verfahren zur Beschleunigung des Wachstums von Haustieren und Geflügel und zur Erhöhung ihrer Futterverwertung, dadurch gekennzeichnet, daß man den Tieren oder dem Geflügel eine wirksame Menge Antibiotikum 76—11 gemäß Anspruch 1 verabreicht.

7. Verfahren zur Herstellung eines Futters für Hausgeflügel, gekennzeichnet durch Verbinden von Antibiotikum 76—11 gemäß Anspruch 1 in einer gegen Kokzidiose des Geflügels wirksamen Menge mit den anderen üblichen Futterb standt ilen.

8. Verfahren nach Anspruch 7, wobei das Antibiotikum 76—11 in einer Konzentration von etwa 5 bis 200 T.p.M. verwendet wird.

9. Verfahren zur Herstellung ines Futters für Haustier oder Geflügel, gekennzeichnet durch

Verbinden von Antibiotikum 76—11 gemäß Anspruch 1 in einer zur Beschleunigung des Wachstums der Tiere oder des Geflügels und zur Erhöhung ihrer Futterverwertung wirksamen Menge mit den anderen üblichen Futterbestandteilen.

10. Verfahren nach Anspruch 9, wobei das Antibiotikum 76—11 in einer Konzentration von etwa 1 bis 200 T.p.M. verwendet wird.

Revendications pour les Etats Contractants; BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Le micro-organisme Actinomadura Sp. 76—11, FERM BP-83.

2. L'antibiotique 76—11 obtenu en cultivant le microorganisme FERM BP 83 et en isolant l'antibiotique 76—11 de la culture.

3. L'antibiotique 76—11, caractérisé par les propriétés physico-chimiques et biologiques suivantes:

(1) Analyse élémentaire:
Acide libre: C: 62,61%; H: 8,27%; N: 0%
Sel de Na: C: 60,57%; H: 8,04%; N: 0%

(2) Poids moléculaire:
843 (mesuré par la méthode de titrage)
873 (mesuré par la méthode de spectre de masse FD)

(3) Point de fusion:
Acide libre: 108—112°C
Sel de Na: 210—212°C (décomposé)

(4) Pouvoir rotatoire spécifique:
$[\alpha]_D^{25}$+36,6° (C=0,382, dans solution de chloroforme)

(5) Spectre d'absorption ultraviolet:
Bandes d'absorption maximales:
Dans MeOH et HCl—MeOH:

λmax=217 mμ ($E_{1cm}^{1\%}$ 303)
262 mμ ($E_{1cm}^{1\%}$ 182)
301 mμ ($E_{1cm}^{1\%}$ 68)

Dans MeOH alcalin:

λmax=260 mμ ($E_{1cm}^{1\%}$ 87)
308 mμ ($E_{1cm}^{1\%}$ 50)

(6) Spectre d'absorption infrarouge:
Bandes d'absorption spécifiques principales dans plaque de KBr:

Acide libre: 3450, 2960, 1720, 1640, 1610, 1578, 1446, 1380, 1315, 1292, 1250, 1209, 1151, 1100, 1035, 975, 940 cm^{-1}
Sel de Na: 3390, 2960, 1718, 1640, 1609, 1578, 1450, 1380, 1340, 1316, 1250, 1197, 1152, 1108, 1092, 1060, 1040, 1002, 980, 930 cm^{-1}

(7) Solubilité dans les solvants:
Alsément soluble dans le benzène, le chloroforme, l'acétate d'éthyle et l'acétone, soluble dans le méthanol, l'éthanol et le diméthylformamide, et difficilement soluble dans l'eau et l'hexane

(8) Réactions de coloration:
Positif à la réaction au permanganate de potassium mais négatif à la réaction à l'acide periodique-benzidine.

(9) Basicité, acidité ou neutralité:
Substance acide, pKa' de 4,6 (dans dioxane à 66,7%)

(10) Couleur:
Cristaux incolores

(11) Activité antimicrobienne:
On a constaté une inhibition de la croissance vis-à-vis des bactéries non-attaquables par les acides, des bacilles et des coccus Gram-positifs en la concentration de 0,4 mcg/ml.

4. Procédé de pr duction de l'antibiotique 76—11 tel que défini dans l s revendicati ns 2 et 3, caractérisé en c qu'il comprend la culture d'un micro-organism pr duisant d l'antibiotique 76—11 appartenant au genre Actinomadura et l'isolem nt de l'antibiotiqu 76—11 des pr duits de culture.

5. Procédé suivant la revendication 4, caractérisé en ce qu le micro-organisme pr duisant de l'antibi tique 76—11 appartenant au genre Actinomadura st le FERM-BP-83.

6. Procédé suivant l'une ou l'autre des revendication 4 et 5, caractérisé en ce que l'on réalise l'isolement en recueillant un extrait exempt de cellules du milieu de culture et en fractionnant l'extrait par un procédé chromatographique de manière à obtenir l'antibiotique 76—11.

7. Agent anticoccidiosique comprenant l'antibiotique 76—11 tel que défini dans les revendications 2 et 3 à titre d'ingrédient efficace.

8. Agent accélérant la croissance et augmentant l'efficacité de l'alimentation pour animaux et oiseaux domestiques comprenant l'antibiotique 76—11 défini dans les revendications 2 et 3 à titre d'ingrédient efficace.

9. Procédé pour accélérer la croissance des animaux et oiseaux domestiques et accroître l'efficacité de leur alimentation, caractérisé en ce qu'il comprend l'administration d'une quantité efficace de l'antibiotique 76—11 défini dans les revendications 2 et 3 aux animaux et oiseaux.

10. Alimentation pour oiseaux domestiques comprenant l'antibiotique 76—11 défini dans les revendications 2 et 3 en une quantité efficace contre la coccidiose des oiseaux.

11. Alimentation suivant la revendication 10, caractérisé en ce que l'antibiotique 76—11 est contenu en la concentration d'environ 5 à 200 ppm.

12. Alimentation pour animaux ou oiseaux domestiques comprenant l'antibiotique 76—11 défini dans les revendications 2 et 3 en une quantité efficace permettant d'accélérer la croissance des animaux ou des oiseaux et d'accroître l'efficacité de leur alimentation.

13. Alimentation suivant la revendication 12, caractérisé en ce que l'antibiotique 76—11 est contenu en une concentration d'environ 1 à 200 ppm.

Revendications l'Etat Contractant; AT

1. Procédé d'obtention de l'antibiotique 76—11, répondant aux propriétés physico-chimiques et biologiques suivantes:

(1) Analyse élémentaire:
Acide libre: C: 62,61%; H: 8,27%; N: 0%
Sel de Na: C: 60,57%; H: 8,04%; N: 0%

(2) Poids moléculaire:
843 (mesuré par la méthode de titrage)
873 (mesuré par la méthode de spectre de masse FD)

(3) Point de fusion:
Acide libre: 108—112°C
Sel de Na: 210—212°C (décomposé)

(4) Pouvoir rotatoire spécifique:
$[\alpha]_D^{25}$+36,6° (C=0,382, dans solution de chloroforme)

(5) Spectre d'absorption ultraviolet:
Bandes d'absorption maximales:
Dans MeOH et HCl-MeOH:

λmax=217 mμ ($E_{1cm}^{1\%}$ 303)
262 mμ ($E_{1cm}^{1\%}$ 182)
301 mμ ($E_{1cm}^{1\%}$ 68)

Dans MeOH alcalin:

λmax=260 mμ ($E_{1cm}^{1\%}$ 87)
308 mμ ($E_{1cm}^{1\%}$ 50)

(6) Spectre d'absorption infrarouge:
Bandes d'absorption spécifiques principales dans plaque de KBr:

Acide libre: 3450, 2960, 1720, 1640, 1610, 1578, 1446, 1380, 1315, 1292, 1250, 1209, 1151, 1100, 1035, 975, 940 cm^{-1}
Sel de Na: 3390, 2960, 1718, 1640, 1609, 1578, 1450, 1380, 1340, 1316, 1250, 1197, 1152, 1108, 1092, 1060, 1040, 1002, 980, 930 cm^{-1}

(7) Solubilité dans les solvants:
Aisém nt soluble dans le benzène, le chloroforme, l'acétate d'éthyle et l'acétone, s luble dans le méthanol, l'éthanol et le diméthylformamide, et difficilement soluble dans l'eau et l'hexane

(8) Réactions de coloration:
Positif à la réaction au permanganate de potassium mais négatif à la réaction à l'acide periodique-benzidine.

(9) Basicité, acidité ou neutralité:
Substance acide, pKa' de 4,6 (dans dioxane à 66,7%)

(10) Couleur:
Cristaux incolores

(11) Activité antimicrobienne:
On a constaté une inhibition de la croissance vis-à-vis des bactéries non-attaquables par les acides, des bacilles et des coccus Gram-positifs en la concentration de 0,4 mcg/ml, caractérisé en ce que l'on cultive un micro-organisme produisant de l'antibiotique 76—11 appartenant au genre Actinomadura et en ce que l'on isole l'antibiotique 76—11 des produits de culture.

2. Procédé suivant la revendication 1, caractérisé en ce que le micro-organisme produisant de l'antibiotique 76—11 appartenant au genre Actinomadura est le FERM-BP 83.

3. Procédé suivant l'une ou l'autre des revendications 1 et 2, caractérisé en ce que l'on réalise l'isolement en recueillant un extrait excempt de cellules du milieu de culture et en fractionnant l'extrait par un procédé chromatographique de manière à obtenir l'antibiotique 76—11.

4. Procédé de préparation d'un agent anticoccidiosique, caractérisé en ce que l'on combine l'antibiotique 76—11 défini dans la revendication 1 à titre d'ingrédient efficace avec des supports et des additifs usuels.

5. Procédé de préparation d'un agent accélérant la croissance et accroissant l'efficacité de l'alimentation pour animaux et oiseaux domestiques, caractérisé en ce que l'on combine l'antibiotique 76—11 défini dans la revendication 1 à titre d'ingrédient efficace avec des supports et additifs usuels.

6. Procédé pour accélérer la croissance d'animaux et d'oiseaux domestiques et pour accroître l'efficacité de leur alimentation, caractérisé en ce qu'il comprend l'administration d'une quantité efficace de l'antibiotique 76—11 tel que défini dans la revendication 1 aux animaux et oiseaux.

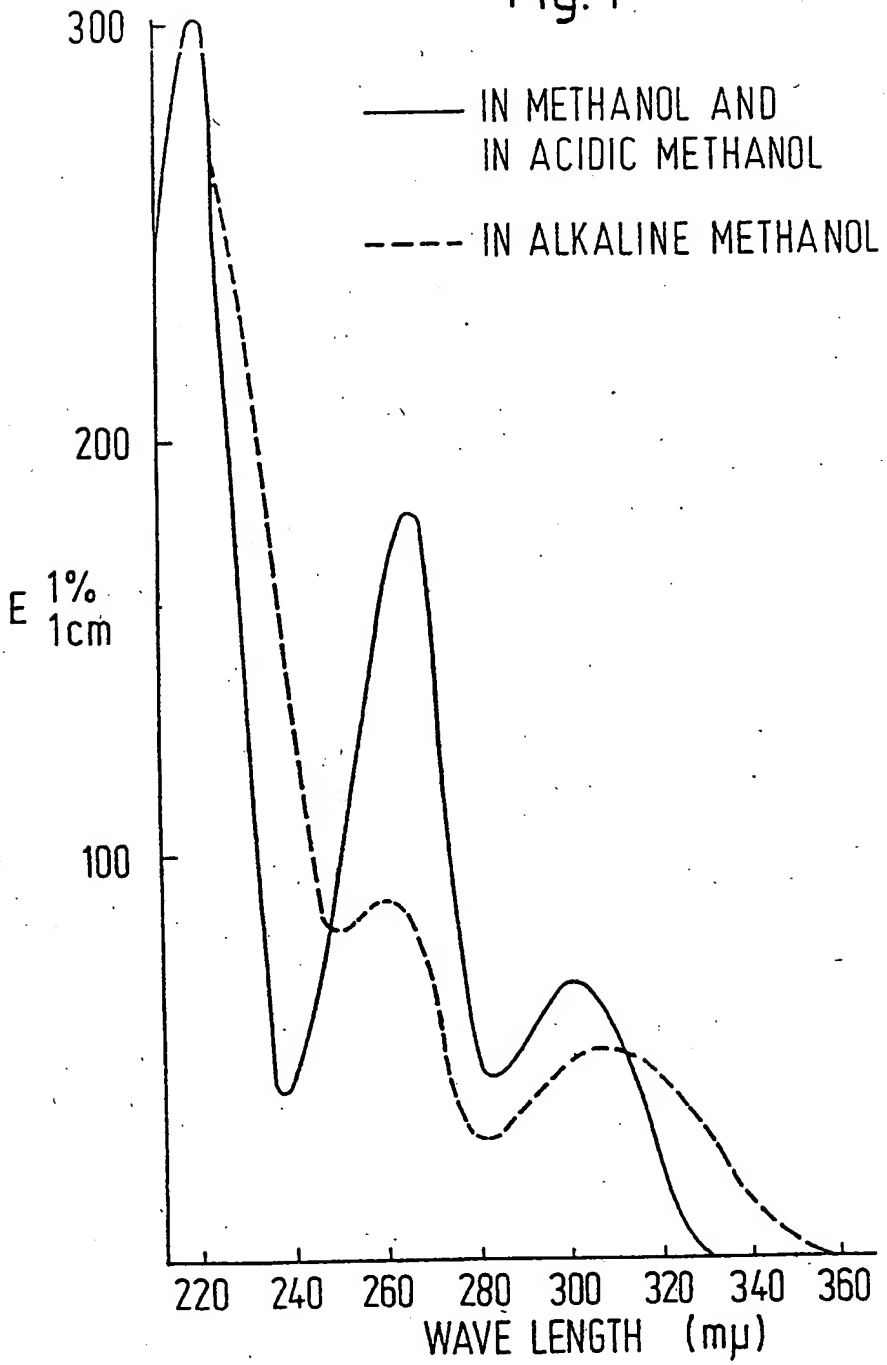
7. Procédé de préparation d'une alimentation pour oiseaux domestiques, caractérisé en ce que l'on combine l'antibiotique 76—11 tel que défini à la revendication 1 en la quantité efficace contre la coccidiose des oiseaux avec lex autres ingrédients d'alimentation usuels.

8. Procédé suivant la revendication 7, caractérisé en ce que l'on utilise l'antibiotique 76—11 en la concentration d'environ 5 à 200 ppm.

9. Procédé de préparation d'une alimentation pour animaux ou oiseaux domestiques, caractérisé en ce que l'on combine l'antibiotique 76—11 tel que défini à la revendication 1 en la quantité efficace pour accélérer la croissance des animaux ou oiseaux et pour accroître l'efficacité de leur alimentation avec les autres ingrédients d'alimentation usuels.

10. Procédé suivant la revendication 9, caractérisé en ce que l'on utilise l'antibiotique 76—11 en une concentration d'environ 1 à 200 ppm.

Fig. 1


300
200
100
E 1% 1cm
IN METHANOL AND IN ACIDIC METHANOL
IN ALKALINE METHANOL
220
240
260
280
300
320
340
360
WAVE LENGTH (mμ)

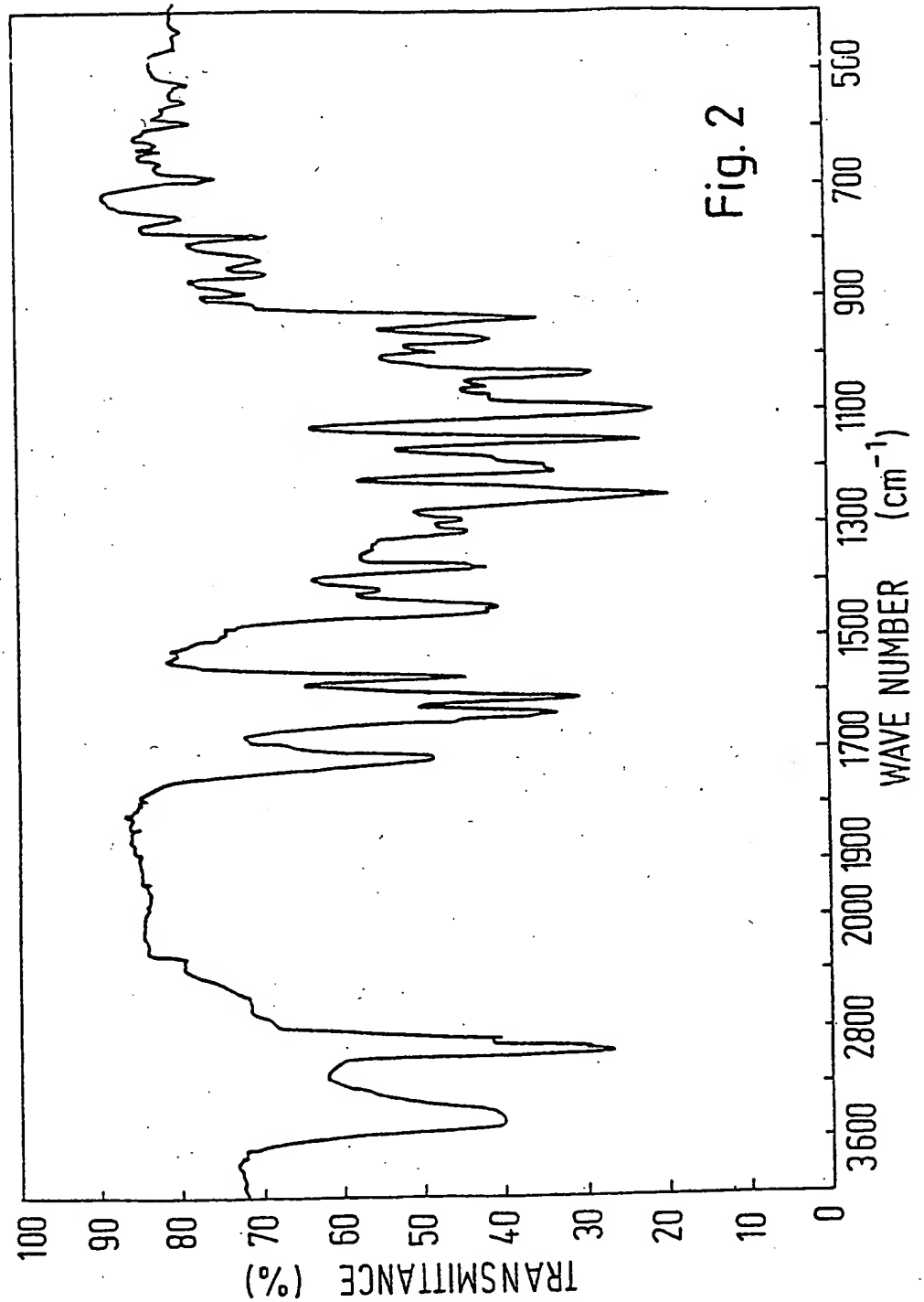

TRANSMITTANCE (%)
100
90
80
70
60
50
40
30
20
10
0
3600
2800
2000
1900
1700
1500
1300
1100
900
700
500
WAVE NUMBER (cm⁻¹)

Fig. 2

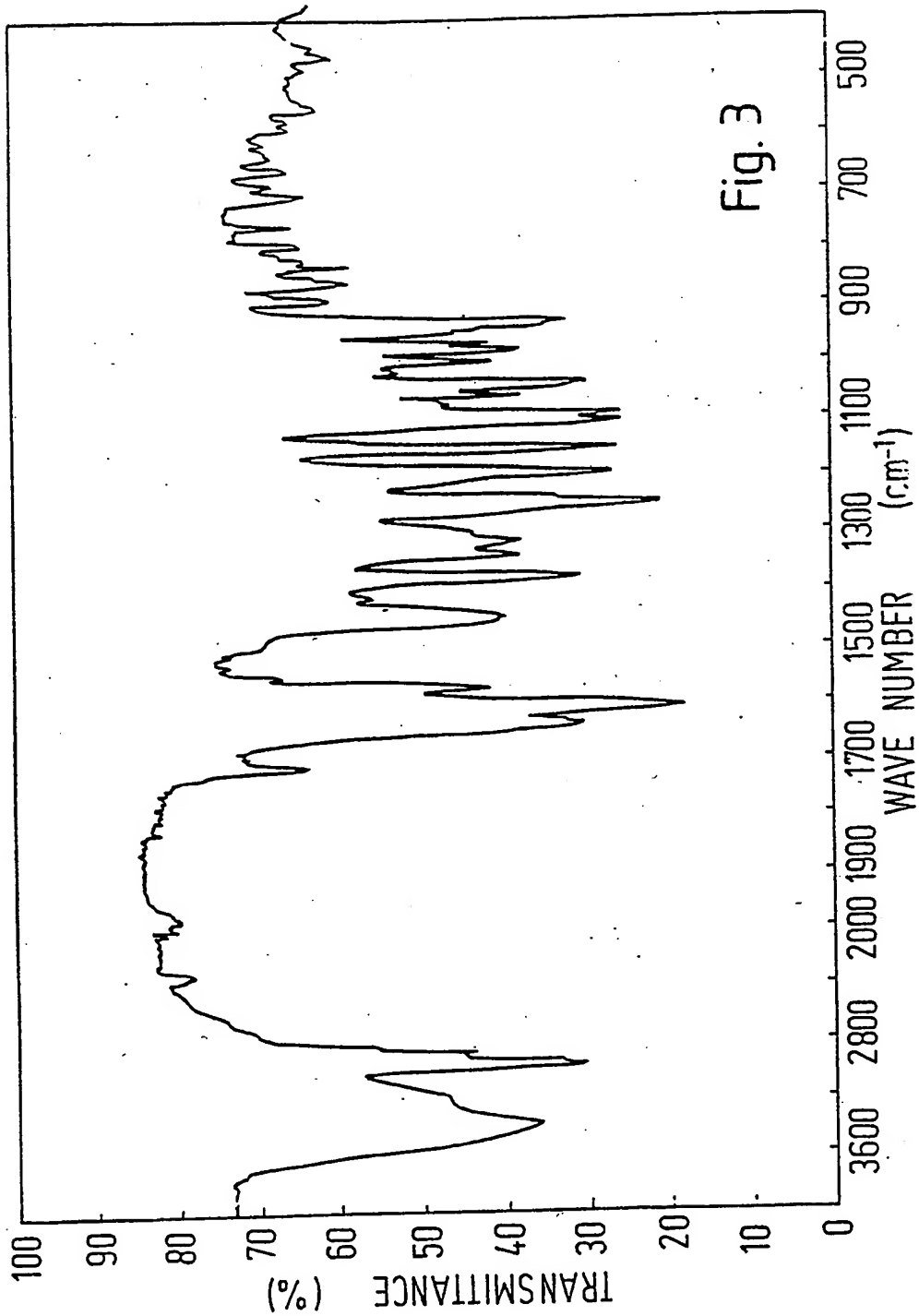

TRANSMITTANCE (%)
100
90
80
70
60
50
40
30
20
10
0
3600
2800
2000
1900
1700
1500
1300
1100
900
700
500
WAVE NUMBER (cm⁻¹)

Fig. 3